The Nature of Arginine Auxotrophy in Cutaneous Populations of Staphylococci

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L-Arginine was required for growth by a high percentage of strains of *Staphylococcus* species that were niche-specific and/or host-specific, but was usually not required for growth by species showing a wide host range. Growth stimulation patterns with arginine intermediates indicated that most of the auxotrophic strains had blocks in an early step(s) in arginine biosynthesis. These strains were designated phenotypically as Arg(CHG) according to the *Salmonella typhimurium* classification scheme. *Staphylococcus simulans* strains appeared to be either ArgA or ArgI. The ArgI strains of *S. simulans* and *S. capitis* had moderate to high ornithine carbamoyltransferase (EC 2.1.3.3) activities and therefore could not be designated as *argI* mutants. ArgI strains in other species had no or very low ornithine carbamoyltransferase activities. All of the natural *Staphylococcus* auxotrophs tested grew in the presence of L-citrulline and had moderate to high argininosuccinase (EC 4.3.2.1) activities. Arginine auxotrophs of species with a wide host range were often capable of reverting to arginine-independent or complete prototrophic growth, whereas auxotrophs of species that tended to be niche-specific and/or host-specific were incapable of reversion to arginine-independence, even in the presence of various mutagens. A relationship between the nature of arginine auxotrophy and habitat is suggested.

INTRODUCTION

Populations of staphylococci isolated from the skin of man and other mammals can be divided into two major groups on the basis of whether or not they require amino acids for growth (Emmett & Kloos, 1975; unpublished results). Staphylococci requiring amino acids for growth demonstrate species-specific or species group-specific patterns. In the majority of species studied to date (e.g. *Staphylococcus aureus*, *S. intermedius*, *S. simulans*, *S. haemolyticus*, *S. warneri*, *S. hominis*, *S. epidermidis* and *S. capitis*) the most commonly essential amino acids are arginine, valine and proline. Amino acids are rarely required by such species as *S. xylosus* and *S. sciuri*, but any such requirement is usually for one of the above amino acids.

Arginine and valine are found in relatively high concentrations (135.8 ± 39.2 and $29.6 \pm 7.5 \mu\text{g ml}^{-1}$, respectively) in normal human sweat that bathes the skin surface (Hier *et al.*, 1946; Farrior & Kloos, 1976). In addition to arginine, citrulline and presumably ornithine (intermediates of arginine biosynthesis) are present on the human skin surface (Rothman & Sullivan, 1949).

In another mammalian host-adapted species, *Neisseria gonorrhoeae*, arginine, proline, and valine are also among the most commonly required amino acids (Catlin, 1976; Young *et al.*, 1977).

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Fig. 1. The arginine biosynthetic pathway of *Salmonella typhimurium* and *Escherichia coli* (Sanderson, 1972; Vogel & Vogel, 1974). *Escherichia coli* locus designations are shown in parentheses.

In the study reported here, we have explored the nature of the arginine requirement (auxotrophy) in various populations of staphylococci isolated from mammalian skin. One of the purposes of this investigation was to identify the blocks in L-arginine biosynthesis responsible for natural auxotrophy in staphylococci. In the biosynthetic pathway, L-glutamate is ultimately converted to L-arginine. *Escherichia coli* (Vogel *et al.*, 1963; Vogel & Vogel, 1974), *Salmonella typhimurium* (Demerec *et al.*, 1960), *Proteus mirabilis* (Prozesky, 1967) and *Bacillus subtilis* (Vogel & Vogel, 1963*a*) utilize the pathway shown in Fig. 1. Our study was also designed to determine whether or not arginine auxotrophy in staphylococci could be induced to revert to prototrophy by various mutagens, in order to gain insight into the genetic nature of the requirement.

METHODS

Bacterial strains. The *Staphylococcus* strains used in this investigation have been classified previously and were isolated from an average of 12 sites on the healthy skin of two subgroups of people, consisting of 20 individuals per subgroup, living in different regions of the U.S.A. (Kloos & Schleifer, 1975; Schleifer & Kloos, 1975) and from the skin of various domestic and wild animals from different areas (Kloos *et al.*, 1976*a, b*). The skin flora from these subgroups and a third subgroup from London (Kloos & Noble, unpublished) did not differ significantly, as was the case for animal species from different regions. Thus, we believe that these strains are representative of the general population of cutaneous staphylococci. The 1059 strains studied are too numerous to list individually; however, the numbers of strains studied of each species and their sources are summarized in Table 1.

Salmonella typhimurium reference arginine auxotrophs *argA55*, *argB51*, *argC113*, *argE72*, *argF87* and *argH93*, derived from strain LT2, were obtained from Dr K. E. Sanderson, University of Calgary, Calgary, Alberta, Canada. *Salmonella typhimurium argI537*, derived from strain LT7, was obtained from Dr J. Roth, University of California, Berkeley, California, U.S.A.

Media and cultural conditions. Growth stimulation of staphylococci by intermediates of the arginine biosynthetic pathway and reversion properties of arginine auxotrophs were tested on a defined agar medium described previously (Emmett & Kloos, 1975), but with the omission of L-arginine. The pH of the medium was adjusted to 7.0 by adding 1 M-NaOH before sterilization. For assays of arginine biosynthetic enzymes, staphylococci were grown in a defined broth medium that differed from the above medium by the omission of Noble agar and the addition of 100 µg L-citrulline ml⁻¹.

Salmonella typhimurium argA, *argB*, *argC*, *argH* and *argI* auxotrophs were grown in medium E broth (Vogel & Bonner, 1956) supplemented with 100 µg L-citrulline ml⁻¹ for assays of arginine biosynthetic enzymes. They

Table 1. Summary of *Staphylococcus* strains and species

No. of strains	Species	Cutaneous source*
45	<i>S. aureus</i>	Man
8	<i>S. intermedius</i>	Man (5)†; dog (2); Squirrel monkey, <i>Saimiri sciurea</i> (1)
160	<i>S. xylosus</i>	Man (98); sheep (22); horse (11); cattle (8); Southern flying squirrel, <i>Glaucomys volans</i> (7); Eastern grey squirrel, <i>Sciurus carolinensis</i> (6); dog (6); pig (1); opossum, <i>Didelphis marsupialis (virginiana)</i> (1)
65	<i>S. cohnii</i> (includes 4 tentative subspecies)	Man (40); Squirrel monkey (14); opossum (10); pig (1)
74	<i>S. saprophyticus</i>	Man
30	<i>S. sciuri</i> subsp. <i>sciuri</i>	Eastern grey squirrel (14); opossum (9); man (5)†; Southern flying squirrel (3); raccoon, <i>Procyon lotor</i> (1); dog (1); sheep (1)
14	<i>S. sciuri</i> subsp. <i>lentus</i>	Goat (9); sheep (5)
21	<i>S. simulans</i>	Man
116	<i>S. haemolyticus</i>	Man (109); pig (5)†; cattle (2)†
41	<i>S. warneri</i>	Man
201	<i>S. hominis</i>	Man
186	<i>S. epidermidis</i>	Man
44	<i>S. capitis</i>	Man
42	<i>Staphylococcus</i> sp. 3	Opossum (15); Eastern grey squirrel (14); Southern flying squirrel (5); raccoon (5); pig (2); sheep (1)
7	<i>Staphylococcus</i> sp. 4	Opossum
5	<i>Staphylococcus</i> sp. 5	Squirrel monkey

* The number of strains isolated from each mammalian host species is shown in parentheses.

† Strains suspected as being transient contaminants which originated by contact with natural hosts.

were grown on medium E agar plates for growth stimulation and reversion analyses. The *argF87* auxotroph was grown on medium E agar and broth as described above, but with 2 µg L-arginine ml⁻¹ instead of L-citrulline.

Stock *Staphylococcus* spp. and *Salmonella typhimurium* cultures were maintained on P agar (Naylor & Burgi, 1956) slopes at 4 °C. *Staphylococci* isolated from skin were transferred (subcultured) one to four times on P agar between the time of their original isolation and use in the nutritional studies reported here. This limited amount of subculturing did not affect the original amino acid requirements or the arginine biosynthetic enzyme activities of 10 randomly selected control strains.

Growth stimulation by arginine intermediates. Several crystals of L-glutamic acid, N-acetyl-L-glutamic acid, N²-acetyl-L-ornithine, L-ornithine, L-citrulline and L-arginine were placed on separate defined agar plates, which had been spread on the surface with approximately 10⁷ colony-forming units (c.f.u.) of either *Staphylococcus* spp. or *Salmonella typhimurium* auxotrophs. Cultures were incubated at 34 °C for 48 h and then examined for evidence of growth stimulation around the area of the bacterial lawn where crystals were absorbed.

Cell extract preparation. Cell extracts of *Staphylococcus* spp. and *Salmonella typhimurium* for enzyme assays were prepared from 200 ml cultures (initial density approximately 5 × 10⁷ c.f.u. ml⁻¹) grown in 500 ml Erlenmeyer flasks for 24 h at 37 °C with shaking at 250 rev. min⁻¹ in a Controlled Environment Incubator Shaker (New Brunswick Scientific Co.). Cells were harvested by centrifuging and washed in 10 ml 0.1 M-potassium phosphate buffer, pH 7.0. Washed cell pellets were resuspended in 0.1 M-potassium phosphate buffer, pH 7.0, containing 2 mM-glutathione (reduced). *Staphylococcus* spp. cell extracts were prepared according to the procedures described previously (Proctor & Kloos, 1973). *Salmonella typhimurium* was disrupted by two passages through a French press (100 MPa). The protein concentration of cell extracts was determined by the method of Lowry *et al.* (1951).

Fig. 2. Percentages of *Staphylococcus* strains requiring arginine for growth.

Enzyme assays. Ornithine carbamoyltransferase (EC 2.1.3.3) activity was assayed by a modification of the procedure of Jones *et al.* (1955) as described by Theil *et al.* (1969). The assay incubation period was 20 min for *Salmonella typhimurium* extracts and 10 or 60 min for *Staphylococcus* spp. extracts.

Argininosuccinase (EC 4.3.2.1) was assayed by a modification of the procedure outlined by Theil *et al.* (1969) in which 10 μ mol K_2HPO_4 , pH 9.5, was used in place of the potassium phosphate buffer, pH 7.5. Enzyme assay mixtures were incubated for 60 min with *Salmonella typhimurium* extracts and 15 min with *Staphylococcus* spp. extracts.

Mutagen-induced reversion of arginine auxotrophy to prototrophy. Single drops (0.05 ml) of aqueous solutions of 1 mg *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (NTG) ml^{-1} , 1.2 g ethyl methanesulphonate (EMS) ml^{-1} , 1 mg diethyl sulphate (DES) ml^{-1} , 1 mg Institute for Cancer Research (ICR)-170G ml^{-1} and 1 mg ICR-191E ml^{-1} were placed on separate defined agar plates, freshly inoculated on the surface with approximately 10^7 c.f.u. of either selected *Staphylococcus* arginine auxotrophs, control *Salmonella typhimurium* arginine or cysteine auxotrophs with previously characterized mutations (K. E. Sanderson, personal communication), or *Micrococcus* cysteine auxotrophs. One control plate, containing defined agar without mutagens, was used to test the spontaneous reversion properties of each auxotrophic strain. Cultures were incubated at 34 °C for 48 h and then examined for the presence of prototrophic (revertant) colonies around the area of the bacterial lawn where the mutagen was absorbed. The resulting arginine-independent colonies were tested for stability of growth properties by serial propagation on defined agar plates.

Chemicals. EMS was obtained from Eastman Organic Chemicals, NTG was from K & K Laboratories, Plainview, N.Y., U.S.A., and lysostaphin was from Schwartz/Mann, Orangeburg, N.Y., U.S.A. ICR-170G and ICR-191E were kindly supplied by Dr Hugh J. Creech, Institute for Cancer Research, Philadelphia, Pa., U.S.A.

RESULTS

Arginine auxotrophy in cutaneous populations of staphylococci

For each *Staphylococcus* species studied, the percentage of strains requiring L-arginine for growth is shown in Fig. 2. In coagulase-positive species and the coagulase-negative

S. simulans and *S. epidermidis* species group, composed of *S. epidermidis sensu stricto*, *S. hominis*, *S. haemolyticus*, *S. warneri* and *S. capitis*, 99 to 100% of the strains required L-arginine. Of the strains of *S. cohnii* subspecies isolated from human skin, 52.5% required L-arginine whereas subspecies of *S. cohnii* isolated from various wild and domestic animals did not require this amino acid. The reason for the marked difference in requirement is unclear, but it could conceivably be related to differences in the availability of L-arginine in human and certain other mammalian cutaneous habitats. Other members of the *S. saprophyticus* species group and *S. sciuri*, including both of its recognized subspecies, were predominantly or entirely composed of strains that did not require L-arginine.

Slightly more than half of the strains of *S. saprophyticus* required L-proline and L-valine for growth (Emmett & Kloos, 1975). Over 95% of *S. sciuri* subsp. *sciuri* and *Staphylococcus* sp. 3 strains and 89% of *S. xylosus* strains were completely prototrophic and able to grow well on a modification of the defined medium (see Methods) containing $(\text{NH}_4)_2\text{SO}_4$ as a sole source of nitrogen.

Growth stimulation by arginine intermediates

The *Salmonella typhimurium* reference arginine auxotrophs showed the growth stimulation patterns that would be expected on the basis of their mutant classification, except that *argB51* was not stimulated by *N*-acetyl-L-glutamic acid. *ArgB51*, blocked at the first step in the biosynthesis of arginine (Fig. 1), was stimulated by *N*²-acetyl-L-ornithine, L-ornithine, L-citrulline and L-arginine; *argC113*, blocked at the second step, and *argH93*, blocked at the third step, were also stimulated by these intermediates; *argA55*, blocked at the fifth step, was stimulated by L-ornithine, L-citrulline and L-arginine; *argI537*, blocked at the sixth step, was stimulated by L-citrulline and L-arginine; whereas *argE72*, blocked at the seventh step, and *argF87*, blocked at the eighth step, were stimulated only by L-arginine.

Table 2 shows the results of testing *Staphylococcus* arginine auxotrophs for growth stimulation by various arginine intermediates. For most species, a high percentage of the strains were Arg(CHG), i.e. blocked early (step 4 and/or earlier) in the biosynthetic pathway leading to L-arginine. These auxotrophs may have single or multiple blocks in the early part of the pathway, the determination of which will require future assays for *N*-acetylglutamate synthase (EC 2.3.1.1), *N*-acetylglutamate kinase (EC 2.7.2.8), *N*-acetylglutamate-5-semialdehyde dehydrogenase (EC 1.2.1.38) and acetylornithine 5-amino-transferase (EC 2.6.1.11) activities. Cross-feeding of auxotrophs could not be demonstrated. *Staphylococcus simulans* was unique in that none of the 21 strains tested were phenotypically Arg(CHG); they were all ArgA or ArgI. These and similar auxotrophs found in other species may also have single or multiple blocks in the early part of the pathway, but our results show that they have, at least, a later block than those found in Arg(CHG) auxotrophs, since they were unable to grow on *N*²-acetyl-L-ornithine and/or L-ornithine. *Staphylococcus capitis* was different in that most strains (68%) were ArgA auxotrophs.

Arginine biosynthetic enzyme assays

Activities of ornithine carbamoyltransferase and argininosuccinase (enzymes catalysing the sixth and eighth steps in L-arginine biosynthesis, respectively) were assayed in reference *Salmonella typhimurium* and *Staphylococcus* arginine auxotrophs.

ArgI designated auxotrophs that are actually *argI* mutants should have insufficient ornithine carbamoyltransferase activity for growth on L-ornithine. The specific activities in reference *Salmonella typhimurium* *argB*, *argC*, *argH*, *argA* and *argF* mutants were between 0.84 and 12 units (mg protein)⁻¹, whereas the *argI* mutant specific activity was only 0.05. The specific activities in representative *Staphylococcus* strains are shown in Table 3. Selected ArgI auxotrophs in the species *S. intermedius*, *S. xylosus*, *S. warneri*, *S. hominis* and *S. epidermidis* had very low or no ornithine carbamoyltransferase activity. This finding is consistent with their inability to grow on L-ornithine, in place of L-citrulline or L-arginine,

Table 2. *Growth stimulation of cutaneous Staphylococcus arginine auxotrophs by arginine intermediates*

Species	No. of strains tested	Auxo-trophic class†	Percent-age of strains in class	Arginine intermediates*					
				Glu	AcGlu	AcOrn	Orn	Cit	Arg
<i>S. aureus</i>	45	Arg(CHG)	78	—	—	+	+	+	+
		ArgA	22	—	—	—	+	+	+
<i>S. intermedius</i>	8	Arg(CHG)	50	—	—	+	+	+	+
		ArgA	38	—	—	—	+	+	+
		ArgI	12	—	—	—	—	+	+
<i>S. xylosus</i>	7	ArgI	57	—	—	—	—	+	+
		Arg(CHG)	29	—	—	+	+	+	+
		ArgA	14	—	—	—	+	+	+
<i>S. cohnii</i> (human subspecies)	21	Arg(CHG)	95	—	—	+	+	+	+
		ArgA	5	—	—	—	+	+	+
<i>S. saprophyticus</i>	2	Arg(CHG)	50	—	—	+	+	+	+
		ArgB	50	—	+	+	+	+	+
<i>S. simulans</i>	21	ArgI	57	—	—	—	—	+	+
		ArgA	43	—	—	—	+	+	+
<i>S. haemolyticus</i>	115	Arg(CHG)	71	—	—	+	+	+	+
		ArgA	29	—	—	—	+	+	+
<i>S. warneri</i>	41	Arg(CHG)	71	—	—	+	+	+	+
		ArgA	24	—	—	—	+	+	+
		ArgI	5	—	—	—	—	+	+
<i>S. hominis</i>	200	Arg(CHG)	94	—	—	+	+	+	+
		ArgA	6	—	—	—	+	+	+
		ArgI	< 1	—	—	—	—	+	+
<i>S. epidermidis</i>	185	Arg(CHG)	81	—	—	+	+	+	+
		ArgA	14	—	—	—	+	+	+
		ArgI	5	—	—	—	—	+	+
<i>S. capitis</i>	44	ArgA	68	—	—	—	+	+	+
		Arg(CHG)	30	—	—	+	+	+	+
		ArgI	2	—	—	—	—	+	+
<i>Staphylococcus</i> sp. 5	5	Arg(CHG)	80	—	—	+	+	+	+
		ArgA	20	—	—	—	+	+	+
<i>Staphylococcus</i> sp. 4	6	Arg(CHG)	100	—	—	+	+	+	+

+, Growth stimulation; —, no stimulation.

* Glu, L-glutamic acid; AcGlu, *N*-acetyl-L-glutamic acid; AcOrn, *N*²-acetyl-L-ornithine; Orn, L-ornithine; Cit, L-citrulline; Arg, L-arginine.

† Phenotypic symbols are according to the scheme used in *Salmonella typhimurium* (Sanderson, 1972). Parentheses around symbols indicate that the exact block in biosynthesis is not known. Furthermore, it is not certain that Arg(CHG) auxotrophs are not also blocked in the first step and therefore lack *argB* gene function or that ArgA and ArgI mutants are blocked in only one step.

and suggests that they are probably *argI* mutants. We were surprised to find, however, that ArgI auxotrophs of *S. simulans* and *S. capitis* had high ornithine carbamoyltransferase activities. The failure of these auxotrophs to grow on L-ornithine may be related to a lack of other enzyme activities (e.g. a specific permease) or regulatory functions. Obviously, we do not consider that *S. simulans* or *S. capitis* ArgI designated auxotrophs are *argI* mutants.

All of the natural *Staphylococcus* arginine auxotrophs grew rapidly in the presence of L-citrulline in place of L-arginine. They would therefore be expected to possess sufficient argininosuccinase activity to convert L-argininosuccinate, derived from L-citrulline, to L-arginine to satisfy growth requirements. Our results indicate that this was the case. Specific activities ranged from 19.1 to 77.1 units (mg protein)⁻¹, depending upon the particular

Table 3. *Specific activity of ornithine carbamoyltransferase in cutaneous Staphylococcus arginine auxotrophs*

Species	Strain	Auxotrophic class*	Specific activity†
<i>S. aureus</i>	HK 41	ArgA	13.9
	AW 100	ArgA	87.5
<i>S. intermedius</i>	DW 258	Arg(CHG)	71.8
	RK 12	ArgA	41.6
	SK 2	ArgA	73.1
	HM 13	ArgA	55.3
	HK 91	ArgI	0.1
<i>S. xylosus</i>	CK 15	Arg(CHG)	629
	RM 383	ArgI	2.4
	EC 323	ArgI	0.5
	EC 325	ArgI	0.0
<i>S. simulans</i>	SM 128	ArgA	389
	LH 303	ArgA	733
	SM 81	ArgA	471
	ATCC 27849	ArgA	286
	KL 299	ArgI‡	236
	AW 232	ArgI‡	231
	JHB 21	ArgI‡	589
	MAW 222	ArgI‡	68.2
<i>S. epidermidis</i>	RM 351	Arg (CHG)	186
	AW 76	ArgI	0.6
	RM 172	ArgI	0.0
	RM 233	ArgI	0.0
	PM 325	ArgI	0.6
	WK 5	ArgI	0.8
<i>S. warneri</i>	JL 202	ArgI	0.4
	SE 3	ArgI	0.8
<i>S. hominis</i>	JL 326	ArgI	1.7
<i>S. capitis</i>	HM 11	ArgI‡	119

* Phenotypic symbols are according to the scheme used in *Salmonella typhimurium* (Sanderson, 1972). Auxotrophic class designations were determined on the basis of growth stimulation patterns by arginine intermediates. Parentheses around symbols indicate that the exact block in biosynthesis is not known.

† Specific activity is expressed as units (mg protein)⁻¹.

‡ ArgI auxotrophic class designation is not consistent with specific activity results.

strain. Attempts to assay other arginine biosynthetic enzymes in staphylococci were unsuccessful, although activities could be demonstrated in specific reference *Salmonella typhimurium* strains.

Reversion properties of arginine auxotrophs

Salmonella typhimurium reference mutants *argC113*, *argF87* and *argH93*, known to contain deletions or multisite mutations within their affected *arg* gene (K. E. Sanderson, personal communication), failed to produce prototrophic revertants with any of the mutagens tested. Mutants *argB51*, *argA55* and *argE72*, containing point mutations, responded to one or more of the alkylating agents NTG, EMS and DES.

The reversion responses of various cutaneous *Staphylococcus* arginine auxotrophs are indicated in Table 4. None of the *S. aureus*, *S. intermedius*, *S. simulans*, *S. warneri*, *S. capitis* or *Staphylococcus* sp. 4 arginine auxotrophs tested demonstrated reversion to arginine-independence, either spontaneously or with mutagen pressure. These species, nevertheless, produced pigment and/or morphologically recognizable mutants when treated with the alkylating agents, indicating mutagen uptake. Species included in the *S. saprophyticus* species group contained a moderate to high percentage of arginine auxotrophs capable of reverting to arginine-independent or complete prototrophic growth.

Table 4. *Reversion of cutaneous Staphylococcus arginine auxotrophs to arginine-independence*

Species	No. of strains tested	Percentage of strains showing spontaneous reversion	Percentage of strains showing reversion in response to alkylating mutagen*
<i>S. aureus</i>	13	0	0
<i>S. intermedius</i>	8	0	0
<i>S. xylosus</i>	7	71	71
<i>S. cohnii</i>	14	29	36
<i>S. saprophyticus</i>	2	50	50
<i>S. simulans</i>	13	0	0
<i>S. haemolyticus</i>	30	13	23
<i>S. warneri</i>	17	0	0
<i>S. hominis</i>	25	8	12
<i>S. epidermidis</i>	34	41	47
<i>S. capitis</i>	14	0	0
<i>Staphylococcus</i> sp. 5	5	40	40
<i>Staphylococcus</i> sp. 4	6	0	0

* Mutagens included *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine, ethyl methanesulphonate and diethyl sulphate.

This property remained stable over the three transfers tested on the defined agar medium lacking L-arginine. The major human species found in the *S. epidermidis* species group, including *S. epidermidis*, *S. hominis* and *S. haemolyticus*, and the major Squirrel monkey species, *Staphylococcus* sp. 5, also found in this group, contained a low to moderate percentage of arginine auxotrophs capable of reverting to arginine-independence. None of the arginine auxotrophs were induced to revert in the presence of the ICR mutagens suggesting that they were not frame-shift mutants. The ICR mutagen preparations were active as they induced reversion to prototrophy in several reference *Micrococcus* cysteine auxotrophs. A few that did not demonstrate spontaneous reversion were induced to revert by one or more of the alkylating mutagens.

DISCUSSION

This preliminary study of the nature of arginine auxotrophy has shown several interesting and perhaps important correlations between biosynthetic capability and habitat. Members of the novobiocin-resistant *S. saprophyticus* species group either do not require arginine or, when they do require this amino acid, can often revert to prototrophy and consequently grow on inorganic nitrogen sources. *Staphylococcus sciuri* does not require L-arginine for growth. The above species have a relatively wide host-range and are found primarily on the skin of mammals lower on the evolutionary scale than Great Apes and man (Kloos *et al.*, 1976*a, b*; Kloos, unpublished results). They have also been isolated from soil and water frequented by mammals and birds and are generally believed to be capable of a rather free-living existence. Prototrophy makes this group of organisms particularly adaptable to environments containing low or perhaps no available organic nitrogen sources. A moderate to large percentage of the arginine auxotrophs produced have retained the ability to revert to arginine-independence or prototrophy. They are, in effect, not irreversibly dependent on the cutaneous environment for an exogenous source of L-arginine. This capability could serve as a homeostatic mechanism enabling the adaptation of these staphylococci to a variety of different and changing environments, containing various amounts of L-arginine, thereby ensuring the continued maintenance and proliferation of the species.

In contrast to the widely distributed species mentioned above, host-specific species or

species with a relatively narrow host range usually required L-arginine and other amino acids for growth. One hypothesis is that host specificity in staphylococci may be, in part, a consequence of the accumulation of specific auxotypes, perhaps originating in isolated cutaneous populations. The advanced development of auxotypes with multiple requirements would tend to 'lock' a population into a niche, from which it could not easily be successfully established elsewhere. Certain auxotypes may have a competitive advantage over prototrophs due to the energy saved in reduced or incomplete biosynthesis of metabolites already in the cutaneous environment.

The failure of niche-specific or host-specific species, such as *S. aureus*, *S. intermedius*, *S. simulans*, *S. warneri*, *S. capitis* and *Staphylococcus* sp. 4, to revert to complete prototrophy is due not only to a lack of reversion from arginine auxotrophy to arginine-independence, but also to the presence of multiple amino acid and vitamin requirements. The lack of reversion from arginine auxotrophy alone appears to be due to the presence of deletions or multisite mutations in one or more arginine loci, as such powerful mutagens as NTG and EMS were unable to induce the production of arginine-independent revertants. Growth stimulation and enzyme studies suggest that the block(s) in L-arginine biosynthesis is before the sixth step in most strains. We believe that such stable fastidious species are highly specialized and perhaps not long ago were rather confined to their respective niches. The natural niche area for these species is generally small (e.g. the nares for *S. aureus*, head for *S. capitis*, or marsupium for *Staphylococcus* sp. 4) and rather isolated (Kloos *et al.*, 1976b). Especially in the case of *S. aureus*, man has intervened and expanded the range of possible habitats supporting growth, for example, by the manufacture and creation of new food products and perhaps by the microbial exchange afforded during the domestication of domestic animals.

It is particularly interesting that the human-specific species *S. epidermidis* and *S. hominis* have, in addition to multiple amino acid auxotrophy, a significant number of strains that are capable of reverting to arginine-independence. Such a capability may allow these species to occupy or colonize a wide range of niches in the human cutaneous ecosystem, which would include those not supplied with adequate levels of L-arginine for growth of auxotrophs. This might be one of the reasons why populations of these species extend over much of the cutaneous surface. Investigations into the availability of amino acids in various niches are required before the mechanisms involved in nutritional adaptation can be clearly elucidated.

The stimulation of growth of *Staphylococcus* strains by N^2 -acetyl-L-ornithine indicates the presence of active acetylornithinase (EC 3.5.1.16) in these organisms rather than ornithine acetyltransferase, an enzyme used in the biosynthesis of L-arginine by *Neurospora* (Vogel & Vogel, 1963b), *Saccharomyces* (De Deken, 1962) and *Chlamydomonas* (Dénes, 1970). Based on our preliminary results, the arginine biosynthetic pathway in *Staphylococcus* spp. appears to be similar to that of *Salmonella typhimurium* and *Escherichia coli*.

Our studies reported here with staphylococci paralleled independent studies on *Neisseria gonorrhoeae* (Ploscowe *et al.*, 1977; Young *et al.*, 1977) with regard to several aspects. These investigators found that the majority (85%) of arginine auxotrophs in *N. gonorrhoeae* were capable of growing on L-ornithine, L-citrulline or L-arginine and were, therefore, suspected as being blocked early in the arginine biosynthetic pathway, like most *Staphylococcus* arginine auxotrophs. Based on recombination properties involving sequential transformational events, they concluded that multiple blocks are common in the arginine biosynthetic pathway of *N. gonorrhoeae*, a situation which we believe also occurs in the most specialized species of staphylococci.

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